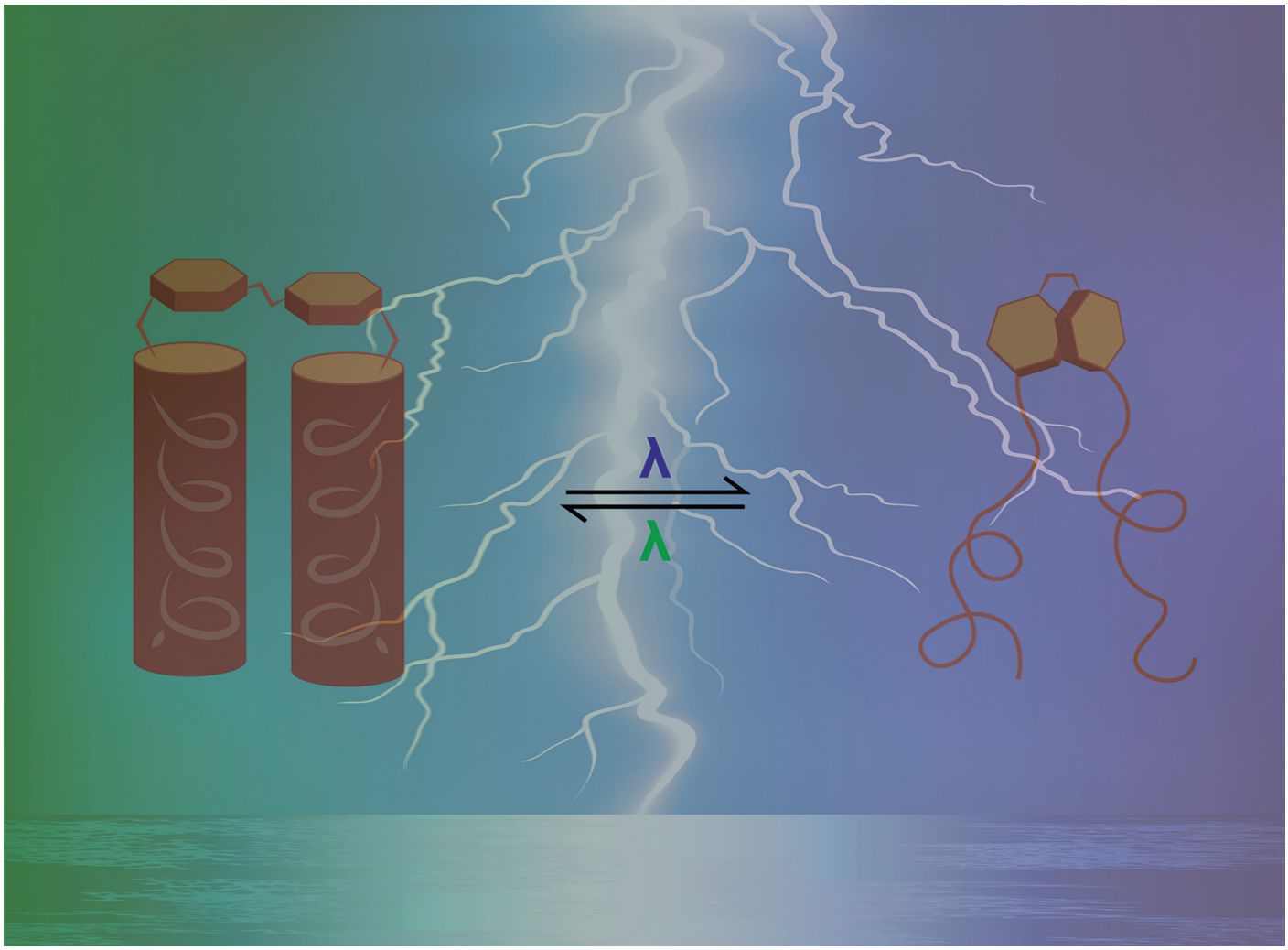

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Conformational control in a photoswitchable coiled coil

Defined conformational photoswitching is exhibited in helical coiled coils. Robust conformational control required precise positioning of a small molecule chromophore across the helical dyad to limit peptide fraying. This research suggests that photoswitchable coiled coils may serve as attractive scaffolds for the development of functional biomaterials.

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Conformational control in a photoswitchable coiled coil†

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The coiled coil is a common protein tertiary structure intimately involved in mediating protein recognition and function. Due to their structural simplicity, coiled coils have served as attractive scaffolds for the development of functional biomaterials. Herein we describe the design of conformationally-defined coiled coil photoswitches as potential environmentally-sensitive biomaterials.

Reversibly controlled peptide assemblies are of interest as dynamic biological reagents and materials.[1–4] Methods described in the literature have utilized redox chemistry,[5,6] pH changes,[7,8] metal coordination,[9–11] and photoisomerization[12,13] to achieve such control. Photoinduced conformational changes in protein secondary structures, α-helices and β-hairpins, have been reproducibly demonstrated;[12–16] however, efforts to control conformation in protein tertiary structures remain in their infancy. The coiled coil motif represents the simplest helical tertiary conformation and attempts to create photoswitchable coiled coils span two decades. The early efforts focused on photocontrol of bZIP transcription factors as a model protein–DNA interaction.[17] Other approaches have involved quaternary structure formation[18] and artificial enzyme modulation.[19] However, robust conformational control, as defined by a significant loss of the helical signature in coiled coil upon irradiation, has not been demonstrated and is the subject of the current study.

We have recently focused on the design of minimal coiled coils as potential inhibitors of protein–protein interactions.[20,21] Natural coiled coils span >30 residues on average and feature a hydrophobic interface which stabilizes the helical conformation in individual segments.[22,23] Our results revealed the challenge in inducing the helical conformations in short peptides that do not have the benefit of extensive interhelical contacts. We learned that judiciously designed crosslinkers can stabilize parallel and antiparallel helical dimers in a predetermined manner.[21] These minimal motifs, termed crosslinked helix dimers or CHDs, have been shown to regulate protein–protein interactions in biochemical, cellular, and *in vivo* contexts.[24] Here, we apply the prior lessons in stabilizing helix dimers to the development of switchable coiled coils.

Small molecule chromophores,[25] such as azobenzene which adopt a *cis* or *trans* conformation upon irradiation with a specific wavelength of light, have been employed to generate photoswitchable protein secondary and tertiary structures.[26] Although significant efforts to achieve defined conformational control have been described, photoswitching to control peptide conformation in coiled coil motifs has been difficult to achieve. The challenge is illustrated in Fig. 1. A stable coiled coil assembly requires interhelical hydrophobic and salt bridge interactions; once these interactions are in place to stabilize the tertiary conformation, azobenzene switching often does not provide the sufficient energy to counteract these interactions. Switching of a stabilized coiled coil, therefore, does not significantly disrupt the peptide helicity.[17,19] This result indicates the propensity of the peptide to adopt a conformation independent of the geometry of the chromophore likely because the individual helices do not act as rigid rods and suffer from helical fraying. We hypothesized that precisely designed crosslinkers and judiciously designed sequence may overcome this challenge and provide defined peptide conformations that can be induced with light.

Coiled coils are characterized by a repeating pattern of polar and non-polar residues known as a heptad repeat (a, b, c, d, e, f, g); a and d positions are generally non-polar in natural coiled coils and drive interhelix *knob into hole* packing.[27] Extensive reengineering of the azobenzene-linked peptide dimer was required to achieve conformationally-defined photoswitching. Our acquired understanding in designing minimal coiled coil peptides stabilized with a crosslinker suggests that a minimum of three-four a/d heptad residues are needed to enable sufficient knob-into-hole packing for helix stability; a higher number of a/d contacts would over-stabilize a coiled coil yet a lower number would likely lead to conformationally unstable constructs. Our study reveals linker trajectory to be a critical factor that must be controlled to afford precise

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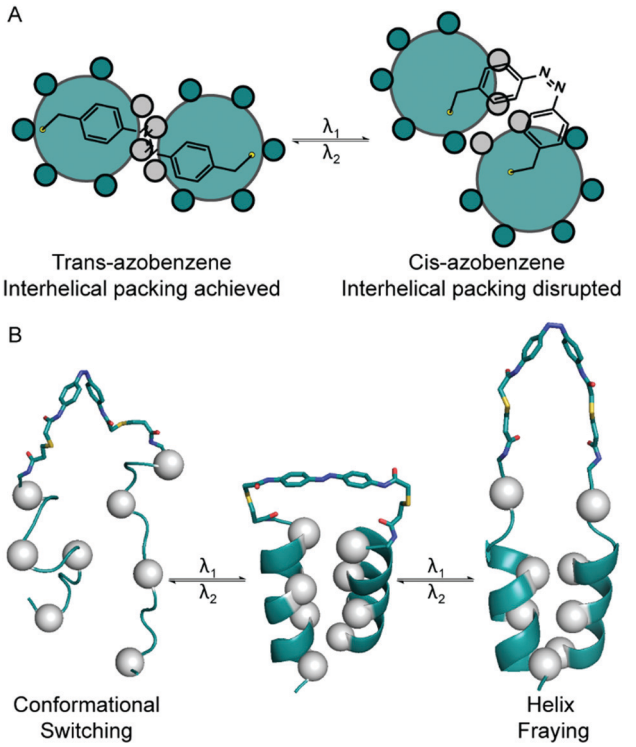
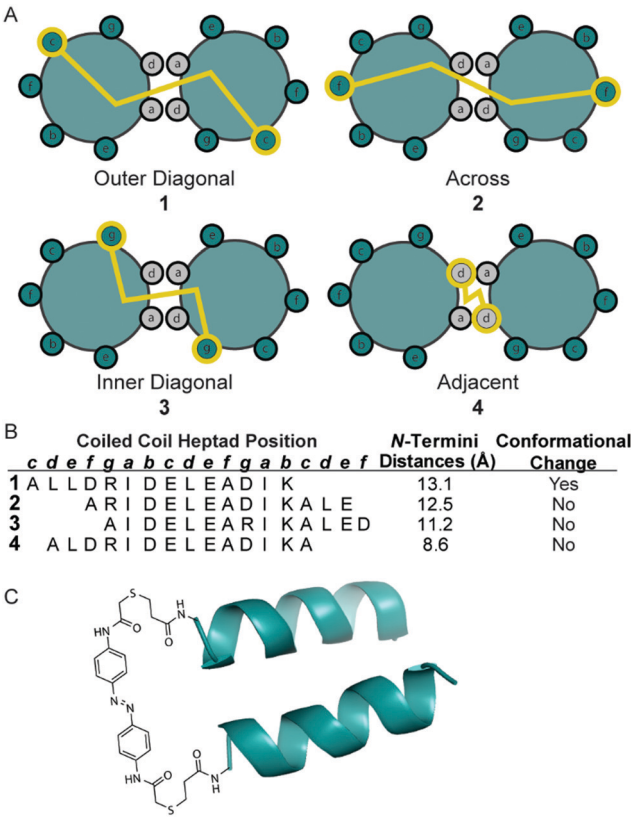


Fig. 1 (A) We developed azobenzene-based coiled coil photoswitches. In our designs, *trans*-azobenzene promotes alignment of hydrophobic residues (grey) and helical assembly while the photo-switched conformation reduces hydrophobic packing. (B) A challenge in developing conformationally-defined coiled coil switches is that individual helices may act as elastic rods and unfold upon conformational switching (left) or undergo partial helix unravelling (right).



B

	Coiled Coil Heptad Position c d e f g a b c d e f g a b c d e f	*N*-Termini Distances (Å)	Conformational Change
1	A L L D R I D E L E A D I K	13.1	Yes
2	A R I D E L E A D I K A L E	12.5	No
3	A I D E L E A R I K A L E D	11.2	No
4	A L D R I D E L E A D I K A	8.6	No

Fig. 2 (A) Potential orientations of azobenzene linkages (yellow) relative to putative helical axes. (B) List of peptide chain sequences aligned by heptad repeat position. (C) Chemical structure of azobenzene linker connected to two peptide strands.

switching. The directionality of the linker *vis a vis* the helix axis is illustrated in Fig. 2A and Fig. S4 (ESI†).

We based the designs of the parallel coiled coil photoswitches on tropomyosin – a well-characterized cytoskeletal coiled coil protein.[28,29] We placed the azobenzene linker at the N-terminus of two peptides to generate parallel coiled coils that span four a/d heptad residues each. We tested four different crosslinking positions at different spots around the helical wheel (Fig. 2B). Peptides **1** through **4** are listed in decreasing N-terminus distances in a putative coiled coil with linkage sites ranging from across the entire structure to directly adjacent (Fig. 2A and B). The N-termini distances in Peptides **1–3** are nearly the same and optimal for the designed azobenzene linker based on our own prior studies[30] and designed helix loop helices.[31] Residues at a and d positions were mutated to Ile and Leu, respectively, for optimal parallel coiled coil hydrophobic packing,[22,32–34] and N-terminal residues were mutated to Ala to maintain consistent and minimal steric bulk in the space adjacent to the chromophore's isomerization. The peptides were synthesized as described in the ESI.† We installed a mercaptopropionic acid residue at the N-terminus of each peptide to afford a thiol group for facile alkylation with 4,4′-dibromoacetamido-azobenzene (Fig. 2C and ESI†).

In designing variants **1–4**, we hypothesized that certain crosslinking sites may appropriately align the a and d position residues for knobs-into-holes packing for coiled coil formation followed by disruption of the helical interface upon photoswitching. The correspondence between the linker length and attachment point is likely to be a factor favouring some designs but our analysis suggests that a second key factor is likely the potential fraying of the peptide strand away from the inner hydrophobic packing (Fig. 1B and Fig. S4, ESI†). In an ideal scenario, the individual α-helices would act like elastic rods that form a dimer or dissociate into monomers in response to the mechanical pressure generated from photoswitching. However, the force may instead lead to fraying of the termini or unravelling of α-turns; we hypothesized that this fraying is the key reason why some coiled coil designs may not display the intended conformational switching.[17,19] Our peptide designs test this hypothesis. We conjectured that attachment of the azobenzene dye at the g/g′ heptad positions (Peptide **3**), is likely not to lead to a conformational change upon photoswitching because the g residues may easily fray; while, attachment of the crosslinker at the c/c′ or f/f′ positions (Peptide **1** and **2**) may allow the individual helices to dissociate upon photoswitching because the presence of two-three residues beyond the helical interface likely provides sufficient stability against helix fraying to engender rod-like behaviour. Using similar logic, we predicted that Peptide **4**, with adjacent crosslinking and a mismatched linker length will not switch conformations.

We modelled several variations and found plausible linker configurations that could accommodate a coiled coil structure envisioned in Fig. 1A and proceeded to assess the designs experimentally. The conformation of the four coiled coil designs was analysed by circular dichroism (CD) spectroscopy. CD spectra of peptides linked to both the *trans*- and the *cis*-azobenzene chromophores were obtained. The *trans*-azobenzene state predominates in the dark; conversion to the *cis*-state was achieved by irradiating the sample with 370 nm light. Peptide **1** exhibited the greatest change beginning with a strong helical signature in the ground state and loss of structure upon irradiation. The magnitude of the change in the helical conformation was probed by the mean residual ellipticity at 222 nm – a signature for α-helices – and a 50% reduction in helicity was observed upon photoswitching (Fig. 3A). To our knowledge, this is the highest amount of photoswitching observed in a coiled coil model. The irradiated peptide reverts back to its higher helical state upon placement of the sample in the dark for 110 minutes at room temperature. As a control, a CD spectrum was also collected for the peptide chain alone without the azobenzene linkage (Fig. S8, ESI†). The control peptide **1c** was less helical highlighting the role of the crosslinker in enforcing the tertiary structure conformation. Peptides **2** and **4** failed to demonstrate a minimal helical signature for either isomer states. In keeping with our predictions, Peptide **3** showed robust helicity but failed to adequately shift conformationally (Fig. 3A and Fig. S7, ESI†). The difference in the behaviour of Peptides **1** and **3** supports the hypothesis that linker heptad position relative to the interface has a significant impact on photoswitching. Specifically, the absence of switching in **3** suggests that helix fraying may be a culprit although it is difficult to directly support helix fraying with NMR structural analysis due to the short lifetime of the *cis*-azobenzene state.

Successful photoisomerization of the azobenzene-linked peptide **1** was confirmed by UV-vis spectrophotometry. Irradiation with 370 nm light led to the disappearance of the *trans*-azobenzene peaks and subsequent onset of those associated with the *cis* state (Fig. 3B). Thermal relaxation in the dark gradually returned the azobenzene to the *trans* state after 110 minutes. To determine the extent of photoisomerization in **1**, we used reversed-phase HPLC and measured areas under the curves for the dark-adapted sample and upon UV-irradiation (Fig. 3C). Data suggest that roughly 90% of the azobenzene-linked peptide undergoes the desired conformational change upon irradiation, which is consistent with previous reports of similarly substituted azobenzene photoswitches.[16]

We examined the oligomerization state of peptide **1** by size exclusion chromatography (Fig. 3D). Lysozyme, aprotinin, and vitamin B12 were analysed as reference standards along with a monomeric crosslinked helix dimer (AB-4) with a solved NMR structure.[21] Peptide **1** eluted with a retention time corresponding to three times the expected molecular weight based on the controls suggesting it exists as a trimer. The high oligomerization state of **1** was corroborated by the concentration-dependent observation of larger particles by dynamic light scattering (Fig. S10, ESI†). No large particle was observed at the

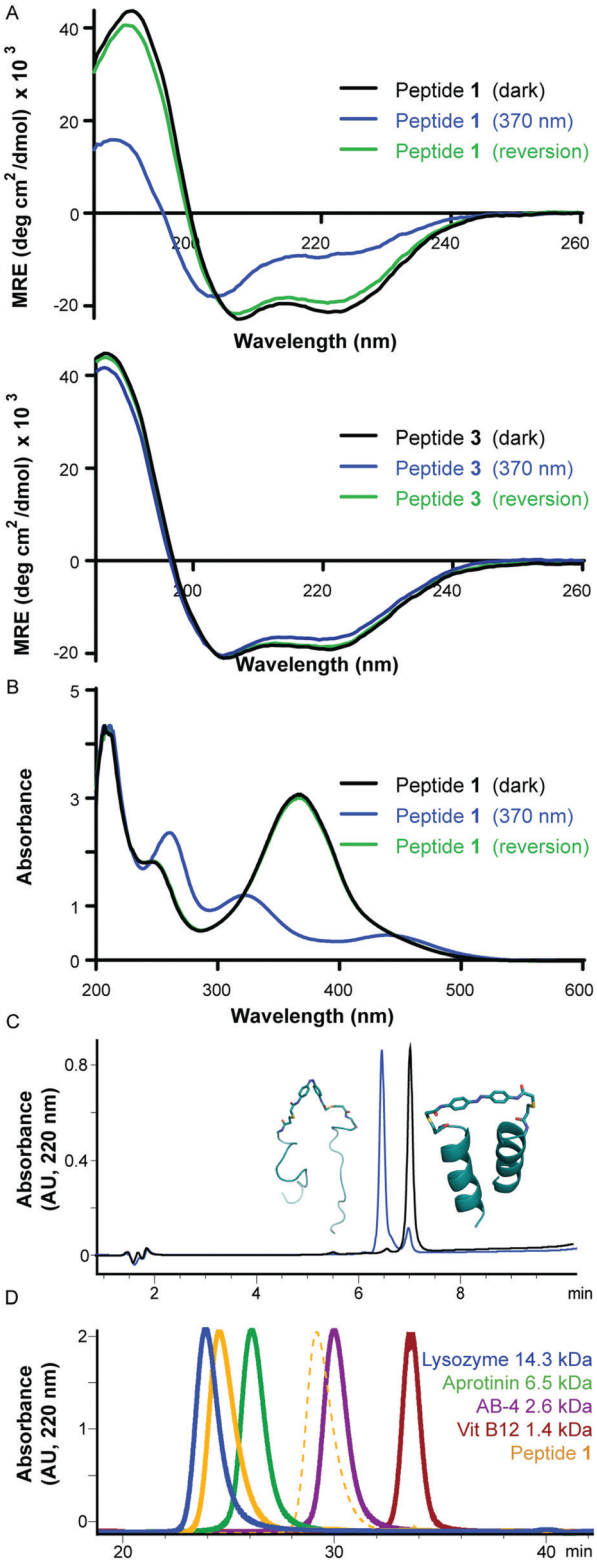


Fig. 3 (A) CD spectra of peptides **1** (top) and **2** (bottom). Black curves are dark-adapted samples and blue curves are after 370 nm irradiation. (B) UV-Vis spectrum of peptide **1**. Dashed black curve is dark-adapted sample, blue curve is after 370 nm irradiation, and green curve is after allowing photoswitch to revert back in the dark. (C) RP-HPLC chromatogram of peptide **1** before (black) and after (blue) photoswitching. (D) SEC chromatograms of peptide **1** (yellow) along with reference standards. Theoretical chromatogram of **1** as a monomer is shown as a dashed line.

concentration (30 μM) used for the CD studies, while a particle size of about 70 nm in diameter was observed at 300 μM, suggesting an oligomer at higher concentrations. Further studies are required to understand the nature of the apparent multimerization.

In conclusion, we report a short, coiled coil peptide construct whose structure can be controlled by irradiation with light. Significant structural change upon photoswitching was achieved, and conformational change did not require binding to a receptor as has been necessary for other efforts to create photoswitchable coiled coils.[17] The data support the postulate that helix fraying from termini is likely a significant factor determining design of robust coiled coil based switches – but that helix fraying may be controlled by the judicious placement of crosslinkers. We anticipate that the strategy presented here may be utilized to develop light-responsive biomaterials.

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Conflicts of interest

There are no conflicts to declare.

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